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Note

Selective interaction of calcium and magnesium ions with ionic dextran derivatives

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Physicochemical studies on ion binding of such cations as Na^+, K^+, Ca^{2+}, and Mg^{2+} to ionic polysaccharides should give a basic information for understanding their roles in ion accumulation and selective ion-binding in biological systems or in food processing. It is of special interest to see how selective cation-binding by ionic polysaccharides is generated by such factors as different ionizable groups, charge density, constituent sugar structure, configuration of the glucosidic linkage, and the resulting flexibility of the polysaccharide chain. From this view point, we have recently elucidated the selectivity of interaction of Na^+ and K^+ ions with ionic dextran derivatives[1,2], through measurements of counterion activity, conductivity, and sound velocity. In this paper, we examine, by measuring counterion activity, selectivity in the binding of Ca^{2+} and Mg^{2+} ions to ionic dextran derivatives carrying $-CH_2CO_2^-$, $-SO_3^-$ and $-PO_3^{2-}$ groups.

The free fraction of divalent counterions, Ca^{2+} and Mg^{2+}, is plotted as a function of equivalent ratio, C_s/C_p, of added divalent cations (C_s) to polyions (C_p) for *O*-(carboxymethyldextran) (**1**), dextran sulfate (**2**), and dextran phosphate (**3**), as shown in Fig. 1. It may be seen that the fraction of free counterions is an increasing function of C_s/C_p with the characteristic shapes of different polysaccharides. With **1**, the fraction of free Mg^{2+} ion is always larger than that of Ca^{2+} ion at a given C_s/C_p ratio, that is, the Ca^{2+} ion binds more strongly to **1** than does the Mg^{2+} ion, and the degree of binding of both cations increases as the degree of substitution of carboxyl groups in **1** increases. It appears that high charge-density causes an increase in the selectivity, whose degree is estimated as $(a^p_{Mg}/a^o_{Mg} - a^p_{Ca}/a^o_{Ca})/(a^p_{Mg}/a^o_{Mg})$ rather than as $(a^p_{Mg}/a^o_{Mg} - a^p_{Ca}/a^o_{Ca})$. Although a rather high polyelectrolyte concentration was used because of limitations in the activity measurements, turbidity or precipitation was never observed in any system incorporating **1**, except for the system of **1** having a degree of substitution of 2.50 in the presence of Ca^{2+} ion at $C_s/C_p > 0.90$. This result suggests that the specific binding between **1** and divalent ions, especially Ca^{2+} ions, is not necessarily only a monomolecular phenomenon, at least at high

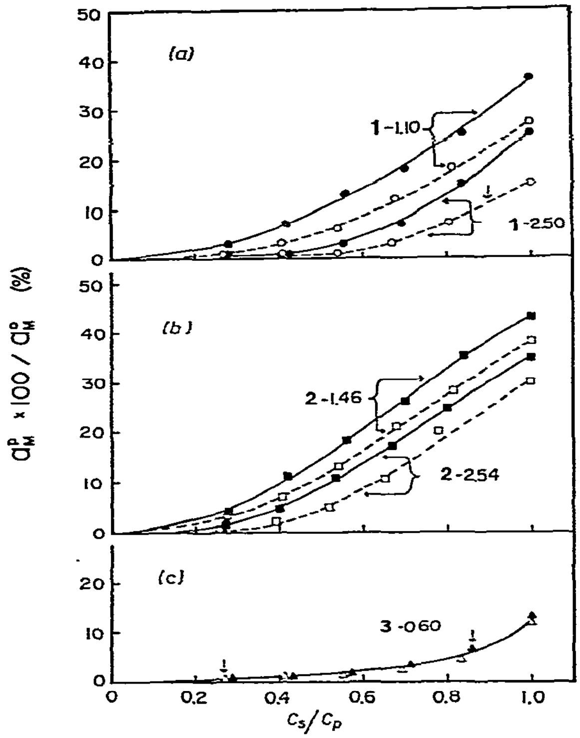

Fig. 1. Plots of free fraction (%) of divalent cations, Mg^{2+} (●, ■, ▲) and Ca^{2+} (○, □, △) as a function of equivalent ratio, C_s/C_p, of added divalent cations (C_s) to polyions (C_p) for ionic dextran derivatives: (*a*) *O*-(carboxymethyl)dextran (**1**), (*b*) dextran sulfate (**2**), and (*c*) dextran phosphate (**3**). The numbers accompanying abbreviated sample names in the figure show the degree of substitution of ionizable groups for each dextran derivative. The mark ↓ above the curves shows the appearance of turbidity.

concentrations of Ca^{2+} ion. It is now generally agreed that the high affinity of Ca^{2+} ion for polycarboxylic ions is, in part, ascribable to the higher chelating ability of Ca^{2+} ions with carboxyl group as compared with Mg^{2+} ions, and also to the difference in their hydrated ion-radii. Thus far, however, no theory of polyelectrolytes is really adequate to introduce the characteristics of the counterions, other than their ionic radii in an electrostatic treatment. Even if a chelated complex may possibly be formed between **1** and Ca^{2+} ion, it would not lead to such strong, interchain cross-links as have been observed in mixtures of alginate and Ca^{2+} ion.

Some investigations have shown that polysaccharides bearing carboxyl group have a stronger affinity for Ca^{2+} than for Mg^{2+} ions. Patel and Patel[3] found that there is a considerable difference in the potentiometric titration-curves of *O*-(carboxymethyl)amylose (**4**) in the presence of magnesium and calcium chlorides. This result may be attributed to a stronger affinity of Ca^{2+} than Mg^{2+} to **4**, although a quantitative analysis was not made. Katchalsky *et al.*[4] reported that the osmotic coefficient for alginate, a mixed polymer of guluronate and mannuronate sequences, is 0.15 for Mg^{2+} and 0.01 for Ca^{2+}, suggesting that there exists a specific, strong binding of Ca^{2+} ion to alginate. Recent circular-dichroism studies have confirmed that alginate forms gels in the presence of Ca^{2+} ions by co-operative association between poly(guluronate) sequences in a regular, buckled-chain conformation of alginate molecule, which sandwich Ca^{2+} ions in an "egg-box" fashion to form microcrystalline, junction zones[5,6]. Rinaudo and Milas[7], however, have reported that the selectivity in divalent-cation interaction is negligible at 25° with *O*-(carboxymethyl)cellulose (5) having various charge-densities, which is clearly different from other polysaccharide derivatives carrying carboxyl groups. Data of ion selectivity for these polysaccharides, alginate, **4**, **1**, and **5**, indicate that not only hydration of counterions but also the constituent sugar structure, the configuration of the glucosidic linkage, and the resulting flexibility of the polysaccharide chain play an important role in generating the selective cation-binding of ionic polysaccharides. Non-selectivity observed for 5 may be attributed to its rigid rod-like structure, as expected from its flexibility parameter[8] and titration data[9].

The interaction behavior of Mg^{2+} and Ca^{2+} ions with **2** seems to be qualitatively similar to that with **1**. In the case of **2**, there was no turbidity at any concentration range of added salts. A noticeable difference between **2** and **1** is that the affinity of both cations with **2** is considerably less than with **1**, and the selectivity is not so great as **1**, although selectivity still exists. This small selectivity could not be found by the precipitation method of Kimizuka *et al.*[10], who showed that, in a 1% solution of 2, no precipitation occurred, even when the concentration of Mg^{2+} or Ca^{2+} ions reached 2M.

The phosphate group introduced into dextran is much more effective in decreasing the fraction of free Mg^{2+} and Ca^{2+} ions than the carboxyl group and the sulfate group, and generates no appreciable selectivity in both types of cation binding, whereas slight turbidity begins to appear at C_s/C_p of 0.27 for Ca^{2+} and 0.85 for Mg^{2+} ions. In the case of **3**, a site binding by electrostatic interaction between divalent anion and divalent cation must be taken into consideration as the cause of strong affinity of divalent cations, as well as the effect of different chelation ability and hydrated ion-radii between both cations. Nevertheless, it is interesting that this 3 shows greater affinity for Na^{+} than for K^{+} ions[11].

EXPERIMENTAL

Materials. — Dextran sulfate (**2**) and *O*-(carboxymethyl)dextran (**1**) were the same as used in earlier work[1]. Dextran phosphate (**3**) was prepared from the purified parent dextran (Meito Sangyo Co. Ltd., Mol. wt. = 30,000) according to the method of Whistler and Towle[12] with slight modifications. The degree of substitution by phosphate group in **3** was determined to be 0.60 by potentiometric titration. Aqueous solutions of these materials were passed through a column of mixed-bed, ion-exchange resin (Amberlite IR-120 and IRA-400) and the acid-form polymers thus obtained were completely neutralized with tetramethylammonium hydroxide (Merck & Co., Inc.) by potentiometric titration. The concentration of polyions was calculated from the titration data.

Activity measurement. — The activity of divalent cations was determined with a divalent cationic electrode, model 92–32 (Orion Research Inc.). A Beckman, frit-junction reference electrode was used. All e.m.f. measurements were made with a Beckman 101900 research pH meter utilizing a 0.2-mV scale. Calibration of the apparatus and the electrode was performed as described in an earlier paper[1]. The tetramethylammonium salt (15 mL) of dextran derivatives (at a concentration of 0.0250 equiv/L) was titrated at 25 ± 0.02° by using standardized 0.250 M calcium chloride or 0.250M magnesium chloride delivered with a microsyringe of 0.2 μL sensitivity, and the activities ($a^{\rm p}_{\rm M}$) of free divalent cations in the solution were measured. According to the same titration-procedure, the activities ($a^{0}_{\rm M}$) of free, divalent cations in 15 mL of distilled water were measured. The degree of binding of cations to the polyions was calculated from the following equation:

$$\text{Degree of binding } (\%) = \frac{a^{0}_{\rm M} - a^{\rm p}_{\rm M}}{a^{0}_{\rm M}} \times 100.$$

thus

$$\text{Free fraction of counterion } (\%) = \frac{a^{\rm p}_{\rm M}}{a^{0}_{\rm M}} \times 100.$$

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